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Effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* among children in São Paulo, Brazil

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ABSTRACT

In March 2010, Brazil introduced the 10-valent pneumococcal conjugate vaccine (PCV10) in the routine infant immunization program using a 4-dose schedule and catch-up for children <23 months. We investigated PCV10 effect on nasopharyngeal carriage with vaccine-type *Streptococcus pneumoniae* (Spn) and non-typeable *Haemophilus influenzae* (NTHi) among children in São Paulo city. Cross-sectional surveys were conducted in 2010 (baseline) and 2013 (post-PCV10). Healthy PCV-naïve children aged 12–23 months were recruited from primary health centers during immunization campaigns. Nasopharyngeal swabs were collected and tested for Hi; for Spn, all baseline and a stratified random sample of 400 post-PCV10 swabs were tested. We compared vaccine-type Spn and NTHi carriage prevalence pre-/post-PCV10, and used logistic regression to estimate PCV10 effectiveness (1-adjusted odds ratio × 100%). Overall 501 children were included in the baseline and 1167 in the post-PCV10 survey (including 400 tested for Spn). Spn was detected in 40.3% of children at baseline and 48.8% post-PCV10; PCV10 serotypes were found in 19.8% and 1.8% respectively, representing a decline of 90.9% ($p < 0.0001$). Carriage of vaccine-related serotypes increased (10.8–21.0%, $p < 0.0001$), driven primarily by a rise in serotype 6C (1.8–11.2%, $p < 0.0001$); carriage of serotypes 6A and 19A did not significantly change. PCV10 effectiveness (4 doses) against vaccine-type carriage was 97.3% (95% confidence interval 88.7–99.3). NTHi prevalence increased from 26.0% (130/501) to 43.6% (509/1167, $p < 0.0001$); PCV10 vaccination seemed significantly associated with NTHi carriage, even after adjusting for other known risk factors. Carriage with PCV10 serotypes among toddlers declined dramatically following PCV10 introduction in São Paulo, Brazil. No protection of PCV10 against NTHi was observed. Our findings contribute to a growing body of evidence of PCV10 impact on vaccine-type carriage and highlight the importance of PCV10 as a tool to reduce the burden of pneumococcal disease in Brazil and globally.

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1. Introduction

Streptococcus pneumoniae (Spn) and *Haemophilus influenzae* (Hi) are important causes of morbidity and mortality worldwide, particularly among young children [1,2]. For each of these organisms, colonization of the nasopharynx is a prerequisite for development of disease, and serves as reservoir for spread [3,4]. Assessments of the prevalence of nasopharyngeal carriage before and after vaccine introduction can be used to evaluate vaccine impact [5]. Changes in carriage following vaccine introduction provide important insight into vaccine impact on invasive disease [6].

Routine use of the *Haemophilus influenzae* type b (Hib) conjugate vaccine has led to dramatic declines in Hib disease and colonization [4]. However non Hi type b serotypes continue to colonize children and can lead to invasive disease [7]. Nontypeable Hi is also frequently found in the nasopharynx [8], and is an important cause of pneumonia, otitis media, sinusitis, conjunctivitis and bacteremia [9,10]. Pneumococcal conjugate vaccines (PCVs) are highly effective against invasive disease and nasopharyngeal colonization caused by pneumococcal vaccine serotypes [11–13] resulting in both direct protection among vaccinated persons as well as herd protection resulting from reduced transmission of vaccine serotypes [14]. However, much of those data are from high-income settings and based on experience with the 7-valent PCV (PCV7). Less is known about PCV impact in low- and middle-income settings and the performance of the newer, high-valent PCVs.

In 2010, Brazil become the first country to introduce the 10-valent PCV (PCV10) nationwide as a routine infant immunization, using a schedule of 3 primary doses (at 2, 4, 6 months of age) plus a booster dose (at 12–18 months) [15]. A catch-up campaign was carried out, which offered 2 primary doses plus a booster for children aged 7–11 months, and a single dose for children aged 12–23 months at the time of introduction [15]. PCV10 contains serotypes 1, 5, 4, 6B, 7F, 9V, 14, 18C, 19F, and 23F. Eight of the serotypes are conjugated to the non-typeable Hi (NTHi) protein D; it has been suggested that PCV10 could reduce otitis media and carriage by NTHi [16,17].

We investigated the effect of PCV10 introduction on nasopharyngeal colonization with pneumococcal vaccine serotypes and NTHi among healthy children living in the biggest metropolitan area in Brazil and explored factors associated with colonization in this context.

2. Materials and methods

2.1. Study area and population

This study comprised two cross-sectional carriage surveys among healthy children aged 12 up to 23 months residing in the municipality of São Paulo. São Paulo is the largest metropolitan region in Brazil, with a population of 11,253,503 inhabitants in 2010, including 710,929 children up to 5 years old [18]. The baseline survey was performed in 2010, and the post-PCV10 survey was conducted in 2013. The participants for both surveys were recruited among children presenting to public Primary Health Units (PHUs) during immunization campaigns carried out on three different dates in 2010 (March 22, June 12 and August 14) and in one in 2013 (June 6).

Children with fever, acute illness or reported antibiotic used during the preceding week were excluded. Only one child per household was enrolled. Children enrolled in the baseline survey were required to have received 3 doses of Hib vaccine (since an objective of that survey was to assess Hib vaccine impact). Some participants in the 2010 survey had received doses of PCV10 (which had been recently introduced) or 23-valent pneumococcal polysaccharide vaccine (used for children with certain chronic diseases) at the time of sample collection; however only those who had not received any pneumococcal vaccine were included in the baseline for this study of PCV10 impact. For the post-PCV10 survey, prior receipt of Hib vaccine or PCV10 were not required and children who had received a pneumococcal vaccine other than PCV10 (i.e. 7-valent PCV or the polysaccharide vaccine) were excluded.

2.2. Sampling and sample size

For the baseline survey, recruitment was conducted at 14 PHUs distributed across the five regions of the metropolitan area (North, n = 2, Central-East, n = 5, East, n = 2, Southeast, n = 5, South, n = 3). For the post-PCV10 survey, children were recruited from the same 14 PHUs as well as 10 additional PHUs (North, n = 4, Central-East, n = 6, East, n = 3, Southeast, n = 6, South, n = 5) in order to enroll the targeted number of children in a single day. The baseline survey included 501 children who had received no pneumococcal vaccination. (A total of 395 children enrolled at baseline had received at least one dose of pneumococcal vaccine and were therefore excluded from this study.) For the post-PCV10 sample size, we took into account the baseline prevalence of carriage with PCV10 serotypes (19.8%) and NTHi (26.0%) among those 501 children. Estimating a reduction of 40% in PCV10-serotypes and 20% of NTHi after PCV10 introduction, we aimed to enroll at least 1320 children for NTHi testing and planned to select 400 of those for testing for Spn. We used a stratified random sample to ensure that the geographic distribution (North, Central-East, East, Southeast, and South) of participants tested for Spn would be the same in the post-PCV10 round as that of the baseline.

2.3. Ethical considerations

The Ethics Committee of the Faculty of Public Health from University of São Paulo (protocol #1969) and the Ethics Committee of Adolfo Lutz Institute (protocol #229.877) approved the surveys carried out in 2010 and 2013, respectively. The protocol for the post-PCV10 survey was reviewed by the Centers for Disease Control and Prevention and determined to be program evaluation and not human subjects research. In both surveys, parent(s)/guardian(s) of participants provided written informed consent before children were enrolled.

2.4. Data and specimen collection

For both surveys, demographic and epidemiologic data were gathered through a standardized questionnaire administered to the parent/legal guardian of participating children before the collection of nasopharyngeal specimens. Vaccination status for PCV10 and Hib was assessed by reviewing the child's immunization card. Doses received at least 14 days before sample collection were counted in the analysis.

In both surveys, a single transnasal nasopharyngeal swab sample per child was obtained by trained nurses using a flexible sterile swab (FLOQ Swabs, Copan) according to WHO working group standard methods [19]. Swabs specimens were immediately inoculated into 1.0 mL skim milk-tryptone-glucose-glycerol transport medium (STGG) [20] and placed in a cold-box and delivered to Center of Bacteriology at Adolfo Lutz Institute (IAL) within 4–5 h after collection.

2.5. Laboratory methods

At IAL, inoculated STGG vials were vigorously vortexed for 20–30 s and then stored at −70 °C. For Spn culture, frozen vials were thawed at room temperature and then vortexed for 20–30 s; 120 μL aliquots were inoculated into 3 mL of TYS broth (Todd-Hewitt broth with 0.5% yeast extract and 600 μL of rabbit serum) for enrichment culture [21]. Spn was identified by standard methods [22] and was serotyped by Quellung reactions with antisera from Statens Serum Institute (Copenhagen, Denmark). All non-typeable pneumococcal isolates by Quellung were confirmed by conventional 8 PCR- multiplex comprising 70 serotypes [21,23]. Penicillin susceptibility was performed by screening for susceptibility to oxacillin by disk diffusion (OXOID, Basingstoke, England), following the CLSI procedure [24]. Spn with diminished susceptibility to penicillin (Oxa halo ⩽ 19 mm) were analyzed for minimum inhibitory concentration (MIC) to penicillin and ceftriaxone by Etest method (bioMerieux, USA) on 5% sheep-blood Muller Hinton agar plate; MIC thresholds conferring resistance to penicillin and to ceftriaxone were considered ⩽0.06 mg/L and ⩽1.0 mg/L, respectively. MIC_{90} and MIC_{50} to penicillin were defined as the MIC value of penicillin at which growth for 90% and 50% of the isolates, respectively, was inhibited.

For Hi culture, 70 μL of inoculated STGG was plated in 10% chocolate agar supplemented with 300 mg/L bacitracin. A suspected colony of *Haemophilus* spp. on chocolate-agar plate was submitted to standard procedures as oxidase test and V (NAD, OXOID) and X (hemin, OXOID) factor requirements to Hi identification [22]. All Hi isolates were confirmed by *hpd*#3 real time PCR (qPCR) assay [25], and were typed by *bexA* [26] and by specific capsule conventional PCR assays [27].

2.6. Data analysis

The primary outcomes were colonization with PCV10 serotypes (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) and colonization with NTHi. Children with more than one pneumococcal serotype detected were classified as being colonized with a vaccine serotype as long as one of the serotypes was vaccine-type. We also examined changes in the prevalence of PCV10-related serotypes, defined as serotypes belonging to the same serogroup as a vaccine serotype (i.e. 6A, 6C, 7C, 9N, 18A, 18B, 19A, 23A, and 23B) and non-PCV10 serotypes (excludes vaccine-related and non-typeable isolates). Spn with negative reactions by Quellung and PCR for serotyping were classified as non-typeable Spn (NT Spn). Prevalence in the baseline and post-PCV rounds were compared using chi-square test and medians compared using Wilcoxon rank sum test; $p < 0.05$ was considered significant. We used logistic regression models to estimate the effectiveness of PCV10 vaccination against vaccine-type pneumococcal colonization and NTHi colonization and to identify risk factors for colonization. Covariates with a p-value < 0.2 on univariate analysis were assessed for potential inclusion in the multivariate models; stepwise automated selection was used to determine which variables remained in the final adjusted models. Vaccine effectiveness was calculated as 1-adjusted odds ratio (aOR) × 100% for PCV10 vaccination. Data analysis was conducted using SAS statistical software (v.9.3).

3. Results

A total of 501 eligible children were included in the baseline (including 477, 8 and 16 enrolled in March, June and August respectively), and 1167 were enrolled in the post-PCV10 survey, of which 400 were selected for Spn testing. The baseline and post-PCV10 participants were similar with respect to age, sex, and number of children aged 0–10 years residing in the household (Table 1). Day care attendance and having ⩾3 people sleep in the same room as the child were significantly less common among baseline participants; low maternal education, reporting a smoker in the household, and having ⩾3 persons aged ⩾11 years in the household were significantly more common among baseline participants compared with the post-PCV10 survey population. The geographic distribution of study participants differed between the baseline and the overall post-PCV10 group, however those selected for Spn testing were similar to baseline. In the post-PCV10 survey, 95.0% of participants had received 3 or 4 doses of PCV10.

Among 501 children in the baseline, 40.3% were colonized with Spn (Table 1), including 5 children with 2 serotypes identified. Among 400 children tested for Spn in the post-PCV10 survey, 48.8% were colonized with Spn ($p = 0.0113$ compared with baseline), including 3 children with 2 serotypes. A total of 33 pneumococcal serotypes were identified among 207 isolates in the baseline survey and 29 serotypes among 198 isolates in the post-PCV10 survey (Fig. 1). Among all isolates at baseline, the most prevalent serotypes were 6B (n = 30, 14.5%), 19F (n = 27, 13.0%), 6A (n = 21, 10.1%), 14 (n = 20, 9.6%), and 23F (n = 16, 7.7%). Post-PCV10 the most common serotypes were 6C (n = 45, 22.7%), 6A (n = 16, 8.1%), and 11A (n = 14, 7.1%). PCV10 serotypes were detected in 19.8% of children in the baseline (Table 1), while the prevalence in the post-PCV10 survey was 1.8%, representing a decline of 90.9% ($p < 0.0001$). The prevalence of carriage of non-PCV10 serotypes increased significantly from baseline to the post-PCV10 survey (8.2% vs. 23.5%, $p < 0.0001$), as did PCV10-related serotypes (10.8% vs. 21.0%, $p < 0.0001$), driven primarily by an increase in serotype 6C (1.8% vs. 11.2%, $p < 0.001$). There was no significant change in the prevalence of carriage of 6A or 19A.

Among 207 pneumococcal isolates from the baseline, 51.7% (n = 107) had a MIC to penicillin > 0.06 mg/L, and 64.5% (n = 69) of those belonged to PCV10-serotypes (serotypes 6B, 14, 19F and 23F); MIC90 and MIC50 to penicillin among the pre-PCV10 isolates were 2.00 mg/L and 0.12 mg/L, respectively. Among 198 isolates from the post-PCV10 survey, 40.4% (n = 80) had MIC to penicillin > 0.06 mg/L, and 7.5% (n = 6) of those were vaccine-serotype; MIC90 and MIC50 to penicillin decreased to 1.00 mg/L and 0.06 mg/L, respectively. Only two isolates from the baseline (serotype 23F and non-typeable) and one isolate from the post-PCV10 survey (serotype 6A) had MIC = 2.0 mg/L for ceftriaxone.

The adjusted effectiveness for three and four doses of PCV10 against carriage by PCV10-serotypes was 92.7% (95% confidence interval [CI] 79.6–97.4) and 97.3% (95% CI 88.7–99.3), respectively (Table 2). Additional factors associated with colonization with PCV10 serotypes were daycare attendance and concurrent colonization with Hi.

Hi was identified in 27.7% of 501 children at baseline and 44.9% of 1167 children in the post-PCV group (Table 1), representing an increase of 62.1% ($p < 0.0001$). The majority of Hi isolates from both the baseline (130/139, 93.5%) and the post-PCV round (509/524, 97.1%) were non-typeable. NTHi carriage increased from 26.0% to 43.6% ($p < 0.0001$). PCV10 vaccination was significantly more common among those colonized with NTHi compared with those not colonized, resulting in a negative adjusted effectiveness (Table 3). Daycare attendance was strongly associated with NTHi colonization (aOR 7.270, 95% CI 5.619–9.405). Additional associated factors included presence of a smoker in the household, low household income, and ⩾2 children aged 0–10 in the house in addition to the study participant.

Table 1
Characteristics, vaccination status and nasopharyngeal colonization among children at baseline and post-PCV10 introduction in São Paulo, Brazil.[a]

	Baseline n = 501 n (%)	Post-PCV10 n = 1167 n (%)	p value[b]	Post-PCV10 subset tested for *Streptococcus pneumoniae* n = 400 n (%)	p value[c]
Characteristics					
Median age in months (IQ Range)	17.0 (6.0)	17.0 (6.0)	0.5471	17.0 (6.0)	0.6563
Male sex	280 (55.9)	589 (50.5)	0.0423	203 (50.8)	0.1244
Attends day care	117 (23.4)	363 (31.1)	0.0013	126 (31.5)	0.0062
Mother educational level ⩽ 8th grade	167 (33.9)	293 (25.3)	0.0003	97 (24.3)	0.0017
Smoker in the household	156 (31.2)	299 (25.6)	0.0191	107 (26.8)	0.1611
Household income ⩽ 1530 R$ per month	272 (64.2)	843 (74.6)	<0.0001	272 (70.6)	0.0492
⩾2 people aged 0–10 years in household [d]	192 (38.3)	498 (42.7)	0.0982	167 (41.8)	0.2966
⩾3 people aged ⩾11 years in household	214 (42.7)	438 (37.5)	0.0468	145 (36.2)	0.0489
⩾3 people sleep in same room as child	111 (22.2)	329 (28.2)	0.0114	113 (28.3)	0.0365
Region					
North	189 (37.7)	271 (23.2)	<0.0001	151 (37.8)	0.9996
South	50 (10.0)	248 (21.2)		39 (9.8)	
East	3 (0.6)	136 (11.6)		2 (0.5)	
Central West	199 (39.7)	269 (23.0)		160 (40.0)	
South East	60 (12.0)	243 (20.8)		48 (12.0)	
Vaccination					
Haemophilus influenzae type b vaccine					
0 doses	0 (0)	29 (2.5)	<0.0001	8 (2.0)	<0.0001
1 dose	0 (0)	7 (0.6)		3 (0.8)	
2 doses	0 (0)	38 (3.3)		9 (2.2)	
3 doses	501 (100.0)	1093 (93.7)		380 (95.0)	
PCV10					
0 doses	501 (100.0)	0 (0)	<0.0001	0 (0)	<0.0001
1 dose	0 (0)	8 (0.7)		6 (1.5)	
2 doses	0 (0)	50 (4.3)		22 (5.5)	
3 doses	0 (0)	488 (41.8)		172 (43.0)	
4 doses	0 (0)	621 (53.2)		200 (50.0)	
Colonization					
S. pneumoniae – any serotype	202 (40.3)	–	–	195 (48.8)	0.0113
PCV10 serotype	99 (19.8)	–	–	7 (1.8)	<0.0001
PCV10-related serotype	54 (10.8)	–	–	84 (21.0)	<0.0001
Serotype 6A	21 (4.2)			16 (4.0)	0.8855
Serotype 6C	9 (1.8)			45 (11.2)	<0.0001
Serotype 19A	9 (1.8)	–	–	10 (5.1)	0.4562
Non-PCV10 serotype[e]	41 (8.2)	–	–	94 (23.5)	<0.0001
Nontypeable	8 (1.6)			10 (2.5)	0.3357
Haemophilus influenzae – any serotype	139 (27.7)	524 (44.9)	<0.0001	175 (43.8)	<0.0001
Type a	2 (0.4)	4 (0.3)	0.8599		
Type b	4 (0.8)	0 (0)	0.0022	–	–
Type d	0 (0)	1 (0.1)	0.5122	–	–
Type e	3 (0.6)	8 (0.7)	0.8410	–	–
Type f	0 (0)	2 (0.2)	0.3538	–	–
Nontypeable	130 (26.0)	509 (43.6)	<0.0001	–	–

[a] Missing values excluded from denominator.
[b] Comparing Phase 1 with Phase 2 overall.
[c] Comparing Phase 1 with Phase 2 subset tested for *S. pneumoniae*.
[d] Refers to children aged 0–10 years in addition to study participant.
[e] Excludes PCV10-related serotypes and nontypeable isolates.

4. Discussion

We observed a robust impact of PCV10 on NP carriage with vaccine serotypes within 3 years of vaccine introduction in Brazil. The prevalence of vaccine-type carriage declined by >90%, and the effectiveness of 3 or 4 doses was >92%. PCV10 also led to a reduction in carriage of penicillin-resistant strains, consistent with the experience with 7-valent PCV [28]. As expected, there was an increase in the prevalence of carriage with non-vaccine serotypes [29]. We also found an increase in vaccine-related serotypes, largely driven by the emergence of serotype 6C as the most common serotype in the post-PCV10 survey. Despite speculation that PCV10 might protect against colonization by NTHi, we observed a significant increase in NTHi carriage following PCV10 introduction and a significant association between PCV10 vaccination and carriage of NTHi.

The impact of PCV introduction on carriage of vaccine serotypes is consistent with results on PCV7 in a variety of settings [13]. However data are more limited on PCV10. A study in Goiania, Brazil conducted soon after PCV10 introduction compared the prevalence of vaccine-type carriage among vaccinated and non-vaccinated children and found the effectiveness of PCV10 (defined as 1-rate ratio ∗ 100%) to be 35.9–44.0% [30]. A study conducted by Hammitt et al. in Kilifi, Kenya found that within 2 years of PCV10 introduction, carriage of vaccine serotypes among all children <5 years had declined by 63% [31]. As with our study, the study by Hammitt was conducted in a setting where a catch-up campaign had been carried out among children <5 years, and where coverage was reasonably high; coverage in Kilifi with at least one dose among children <5 years was 79% in 2012. Because most low- and middle-income countries introduce PCV with no (or only limited) catch-up, and coverage in many settings is lower than

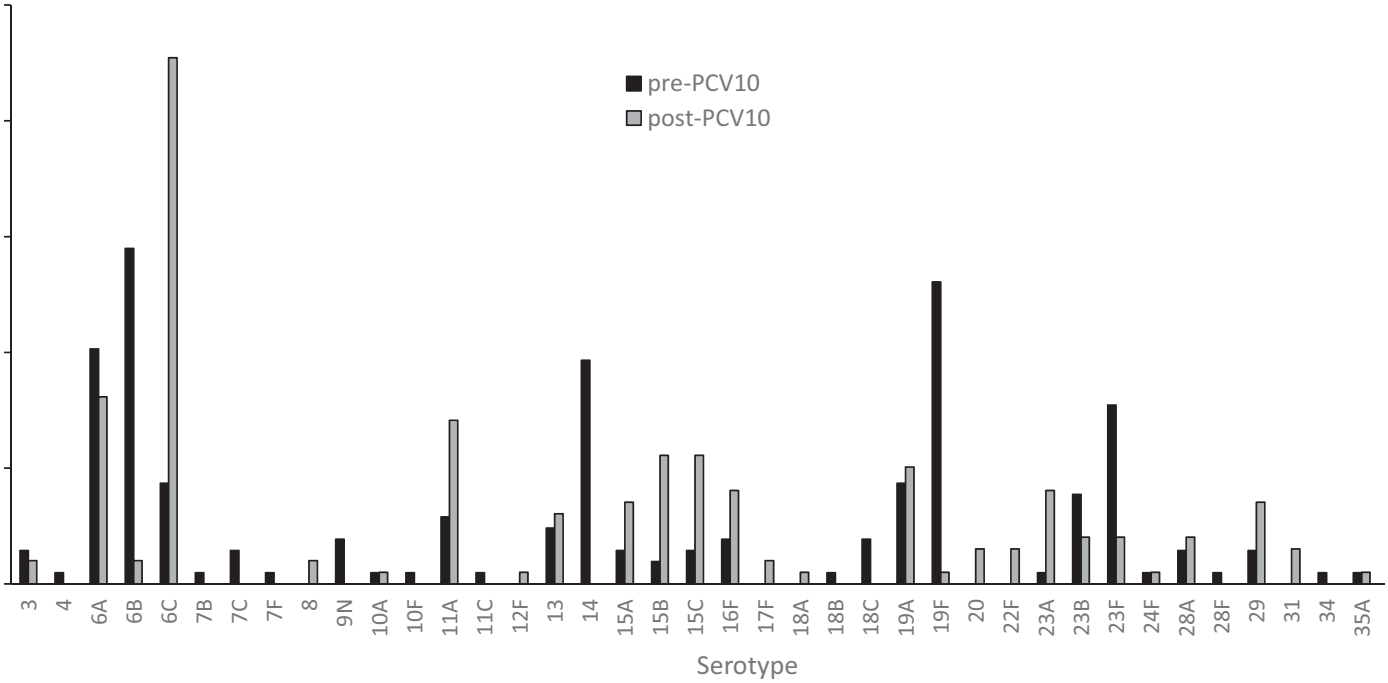

Fig. 1. Serotype distribution among pneumococcal carriage isolates before (n = 207) and after (n = 198) PCV10 introduction.

Table 2
Factors associated with colonization with vaccine-type *Streptococcus pneumoniae*.

	Colonized n = 106 n (%)	Not colonized n = 795 n (%)	Crude odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	PCV10 effectiveness (95% CI)
Age < 18 months	45 (42.4)	439 (55.2)	0.598 (0.397, 0.901)	0.0140	–	
Male sex	57 (53.8)	426 (53.6)	1.008 (0.671, 1.513)	0.9708	–	
Attends day care	46 (43.4)	197 (24.8)	2.327 (1.535, 3.530)	<0.0001	2.358 (1.455, 3.821)	
Mother educational level ⩽ 8th grade	41 (39.8)	223 (28.3)	1.675 (1.097, 2.560)	0.0170	–	
Smoker in the household	38 (35.8)	225 (28.3)	1.413 (0.923, 2.164)	0.1113	–	
Household income ⩽ 1530 R per month	64 (71.1)	480 (66.8)	1.226 (0.757, 1.984)	0.4076	–	
⩾2 people aged 0–10 years in household (in addition to participant)	43 (40.6)	316 (39.8)	1.035 (0.685, 1.564)	0.8714	–	
⩾3 people aged 11 years or older in household	48 (45.3)	311 (39.1)	1.288 (0.856, 1.937)	0.2242	–	
⩾3 people sleep in same room as child	28 (26.4)	196 (24.8)	1.092 (0.688, 1.731)	0.7095	–	
Region						
North	53 (50.0)	287 (36.1)	Ref			
South	9 (8.5)	80 (10.1)	0.609 (0.288, 1.288)	0.1945	–	
East	0 (0)	5 (0.6)	–	0.9880	–	
Central West	34 (32.1)	325 (40.9)	0.567 (0.358, 0.896)	0.0152	–	
South East	10 (9.4)	98 (12.3)	0.553 (0.271, 1.128)	0.1033	–	
Colonization with *Haemophilus influenzae*	53 (50.0)	261 (32.8)	2.046 (1.360, 3.078)	0.0006	2.454 1.529 3.939	
PCV10 doses[a]						
0	99 (93.4)	402 (50.8)	Ref		Ref	
1	0 (0)	6 (0.8)	–	0.9866	–	
2	1 (0.9)	21 (2.6)	0.193 (0.026, 1.455)	0.1105	0.122 (0.016, 0.951)	87.8 (4.9, 98.4)
3	4 (3.8)	168 (21.1)	0.097 (0.035, 0.267)	<0.0001	0.073 (0.026, 0.204)	92.7 (79.6, 97.4)
4	2 (1.9)	198 (24.9)	0.041 (0.010, 0.168)	<0.0001	0.027 (0.007, 0.113)	97.3 (88.7, 99.3)

[a] Reference is 0 doses.

that of Kilifi or São Paulo, the impact of vaccine introduction on vaccine-type carriage in other settings may not be as rapid.

We found no protection of PCV10 against carriage of vaccine-related serotypes. Pre-licensure immunogenicity data suggested that PCV10 might offer cross-protection against vaccine-related serotypes 6A and 19A [16,32]. A study from Finland reported a significant decline in invasive disease caused by both of these vaccine-related serotypes following introduction of PCV10 as a routine infant immunization [33]. A case-control study of PCV10 effectiveness against invasive pneumococcal disease carried out in 10 states of Brazil (including São Paulo) found the vaccine to be significantly protective against vaccine-related serotype 19A [15,32]. Despite such evidence of cross-protection against invasive disease, in this study there was no evidence of PCV10 impact against carriage of either serotype 6A or 19A. This finding is consistent with the carriage study conducted in Kilifi [31], and an observational study from the Netherlands [34], where no effect of PCV10 was observed against vaccine-related serotypes. In contrast, a recent trial from Finland reported a significant reduction in carriage of serotype 19A among children receiving a 4-dose (3 + 1)

Table 3
Factors associated with colonization with non-typeable *Haemophilus influenzae*.

	Colonized n = 639 n (%)	Not colonized n = 1029 n (%)	Crude odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	PCV10 effectiveness (95% CI)
Age < 18 months	339 (53.05)	581 (56.46)	0.871 (0.715, 1.062)	0.1734	–	
Male sex	327 (51.17)	542 (52.67)	0.942 (0.773, 1.147)	0.5513	–	
Attends day care	325 (50.86)	155 (15.06)	5.836 (4.634, 7.351)	<0.0001	7.270 (5.619, 9.405)	
Mother educational level ⩽ 8th grade	181 (28.68)	279 (27.38)	1.067 (0.856, 1.331)	0.5644	–	
Smoker in the household	195 (30.56)	260 (25.27)	1.302 (1.045, 1.621)	0.0184	1.357 (1.047, 1.759)	
Household income ⩽ 1530 R per month	463 (77.04)	652 (68.42)	1.549 (1.226, 1.957)	0.0002	1.733 (1.322, 2.271)	
⩾2 people aged 0–10 years in household (in addition to participant)	317 (49.61)	363 (36.1)	1.731 (1.417, 2.116)	<0.0001	1.805 (1.425, 2.286)	
⩾3 people aged 11 years or older in household	228 (35.68)	424 (41.21)	0.792 (0.646, 0.971)	0.0247	–	
⩾3 people sleep in same room as child	188 (29.42)	252 (24.56)	1.280 (1.026, 1.598)	0.0289	–	
Region						
North	173 (27.07)	287 (27.89)	Ref		–	
South	126 (19.72)	172 (16.72)	1.215 (0.903, 1.636)	0.1987	–	
East	51 (7.98)	88 (8.55)	0.961 (0.649, 1.425)	0.8446	–	
Central West	173 (27.07)	295 (28.67)	0.973 (0.746, 1.269)	0.8395	–	
South East	116 (18.15)	187 (18.17)	1.029 (0.763, 1.387)	0.8508	–	
PCV10 doses[a]						
0	130 (20.34)	371 (36.05)	Ref		Ref	
1	5 (0.78)	3 (0.29)	4.756 (1.121, 20.181)	0.0344	5.400 (1.136, 25.678)	−440 (−2467.8, −13.6)
2	24 (3.76)	26 (2.53)	2.634 (1.461, 4.751)	0.0013	2.001 (1.010, 3.966)	−100.1 (−296.6, −1.0)
3	208 (32.55)	280 (27.21)	2.120 (1.621, 2.773)	<0.0001	2.176 (1.586, 2.986)	−117.6 (−198.6, −58.6)
4	272 (42.57)	349 (33.92)	2.224 (1.724, 2.870)	<0.0001	2.078 (1.536, 2.812)	−107.8 (−181.2, −53.6)

[a] Reference is 0 doses.

schedule of PCV10, but no significant decline in those receiving a 3-dose (2 + 1) schedule [35]. Further study of the impact of PCV10 on vaccine-related serotypes is needed. It is possible that protection against carriage requires higher antibody levels than protection against invasive disease. Because impact against carriage is so important for developing indirect effects [14], it will be important to monitor disease trends for serotypes 6A and 19A in countries using PCV10.

Serotype 6C is also in the same serogroup as vaccine-type 6B, yet we found a significant increase in the prevalence of 6C carriage following PCV10 introduction. PCV7 does not provide cross protection against 6C [36,37], and increases in 6C carriage were observed following PCV7 introduction [38]. Serotype 6C has been found to be relatively non-invasive compared with other serotypes [39]. However given its emergence as the most common colonizing serotype in the post-PCV10 phase, it will be important to monitor for any increases in 6C invasive disease.

Despite speculation that PCV10 could lead to a decline in NTHi pneumonia and otitis media mediated through a reduction in carriage [16], in our study NTHi carriage increased significantly post-PCV10, and PCV10 vaccination was strongly associated with carriage even after adjusting for other risk factors. A clinical trial of an 11-valent precursor to PCV10 carried out in the Czech Republic and Slovakia demonstrated efficacy against otitis media caused by NTHi [40] and against Hi colonization [41], although not statistically significant when molecular methods were used in addition to standard microbiologic techniques. Subsequent clinical trials of PCV10 in the Czech Republic [42], the Netherlands [43], Kenya [44] and Finland [35] found no consistent impact on NTHi carriage. Following PCV10 introduction in Kenya, a significant decline in NTHi carriage was observed in Kilifi when comparing baseline (2009–2010) to post-PCV10 years (2011–2012); however NTHi carriage increased from 2011 to 2012, and vaccination with 2 or more doses (compared with 0 or 1 dose) of PCV10 was not significantly protective against NTHi carriage [31]. Based on available data it was not surprising that we found no protection against NTHi carriage; however the reason for the observed increase in NTHi carriage and significant association between PCV10 vaccination and NTHi carriage is unknown. Because baseline specimens were collected predominantly in the month of March and post-PCV10 specimens in June, it is possible that seasonal differences in NTHi carriage impacted the results. It is also possible that the increase in NTHi may be related to the observed increase in overall pneumococcal carriage, since carriage with one has been positively associated with carriage of the other [45]. The relationship between bacteria in the nasopharynx is complex [46], and it is unclear whether an increase in NTHi might translate into an increase in NTHi disease. Further study of the short- and long-term impact of PCV10 on NTHi carriage is needed.

Beyond vaccination, we identified additional risk factors for Spn and NTHi carriage. Attending day care was a risk factor common to both; this finding is consistent with multiple studies in a variety of settings [47–49]. Adequate vaccination is a requirement for enrollment in day care centers in Brazil, although one study found that 10.9% of 18-month olds enrolled in day care in São Paulo were incompletely vaccinated [50]. For NTHi, we also found that low income, multiple children in the house in addition to the study participant, and having a smoker in the household were risk factors for carriage. These findings are consistent with known risk factors for pneumonia [51], and highlight the importance of multifaceted strategies for reducing the risk of respiratory disease in children. Vaccination with PCV10 is an extremely important tool, but other prevention efforts are needed, particularly to reduce NTHi disease.

This study had several limitations. Participants were a convenience sample recruited from vaccine centers during campaigns, and the study was conducted in a single city, both of which limit generalizability. PCV10 had recently been introduced at the time of the baseline survey; we excluded children who had received PCV10, however it is possible that early indirect effects may have affected the observed prevalence of vaccine-type carriage at baseline. As noted above, the baseline specimens and post-PCV specimens were not all collected at the same time of year; therefore seasonal variations in carriage may have impacted the results. Detection of Spn was done by culture, which is less sensitive than PCR, so we may have failed to detect some pneumococcal carriage or carriage with multiple serotypes. Certain vaccine serotypes, such as 1 and 5, are uncommonly found in carriage despite being important causes of disease [52] so carriage studies do not provide data on PCV10 impact against those serotypes. Furthermore, serotypes 4, 7F and 9V were either rare or absent in this setting, so our

estimate of PCV10 protection against vaccine-type carriage was driven only five serotypes (6B, 14, 18C, 19F and 23F). Finally, other non-vaccine factors can influence carriage prevalence over time; we attempted to adjust for potential confounders in our calculations of vaccine effectiveness, however there may be other factors which we did not account for.

Despite these limitations, we show a clear impact of PCV10 on carriage of vaccine serotypes within 3 years after vaccine introduction. These data add to the growing evidence base of PCV10 impact and effectiveness in Brazil [30,53–55] and elsewhere [31,33,56,57], and can help guide policy decisions. We showed no protection against carriage of vaccine-related serotypes, and increases in NTHi warrant further monitoring. Yet our findings support the use of PCV10 to reduce the burden of pneumococcal disease in Brazil and globally.

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Conflict of interest

MCCB has received lecture fees and travel grants from GlaxoSmithKline and Pfizer; APSL has received lecture fees from Sanofi-Pasteur and Novartis; MCG has received travel grants from Novartis; ALA has received consulting fees and travel grants from GlaxoSmithKline and Pfizer; SCGA has received travel grants from GlaxoSmithKline; TRMPC and AFR has received travel grants from Sanofi-Pasteur. JRV, MGC, RCZ, APB, SB, MLLSG, LSP, HKS, APSS, BL, MLN and MTC have no conflict of interest. All authors have approved the final article.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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